Frequent BRAF V600E mutation in keratocystic odontogenic tumor

Eunae Sandra Cho

The Graduate School
Yonsei University
Department of Dentistry

Frequent BRAF V600E mutation in keratocystic odontogenic tumor

Directed by Professor Jong Hoon Choi

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Eunae Sandra Cho

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연세대학교
YONSEI UNIVERSITY

This certifies that the doctoral dissertation of
Eunae Sandra Cho is approved.

Thesis Supervisor: Jong Hoon Choi

Jong In Yook

Hyung Joon Ahn

Hyun Sil Kim

Jeong Seung Kwon

The Graduate School
Yonsei University
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DEDICATIONS

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Frequent BRAF V600E mutation in keratocystic odontogenic tumor

Eunae Sandra Cho

Department of Dentistry

The Graduate School, Yonsei University

(Directed by Professor Jong Hoon Choi)

Keratocystic odontogenic tumor (KCOT) is a benign intraosseous odontogenic epithelial tumor in the jaw, characterized as an odontogenic cyst, odontogenic keratocyst, in the past. In 2005, the World Health Association working group

reclassified the cyst as a benign tumor for several clinical and molecular genetic reasons.

Clinically, KCOT shows aggressive growth patterns and high recurrence rates which is relatively similar to odontogenic tumors rather than odontogenic cysts. Molecular genetic features present in KCOT support its neoplastic behavior as well, such as proliferative markers and tumor suppressor gene mutations/loss of heterozygosity. Although PTCH gene mutation was considered to be critical in KCOT pathogenesis due to its close association in syndrome related lesions, there are needs in identifying other oncogenic mutations, especially in sporadic KCOT.

BRAF V600E mutation has been identified in odontogenic tumors, representatively ameloblastoma. It is an oncogenic driver mutation which activates the mitogen-activated protein kinase (MAPK) pathway and has commercial target inhibitors, such as vemurafenib and dabrafenib.

In this study, frequent BRAF V600E mutation was identified in KCOT by gene sequencing. The results are as follows.

1. BRAF V600E mutations were identified for the first time in 63.2% of KCOT and 28.6% of OOC by sanger sequencing in formalin fixed paraffin embedded tissue samples with/without microdissection.

2. There was no statistically significant difference between KCOT mutation status and clinical information such as age, sex, location, recurrence and multiple lesions.

3. The gold standard to identify BRAF V600E mutation in KCOT is gene sequencing rather than immunohistochemistry.

4. Microdissection is useful in validating mutations in cystic tumors or inflamed lesions.

The results support KCOT as a tumor and relates its pathogenesis to BRAF V600E mutation and the MAPK pathway. BRAF target inhibitors are expected to be an effective and non-invasive future therapeutics for KCOT in the future.

Key words : Keratocystic odontogenic tumor, BRAF V600E, mitogen-activated protein kinase pathway, vemurafenib, dabrafenib

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I. Introduction

Keratocystic odontogenic tumor (KCOT) is a benign intraosseous odontogenic epithelial tumor in the jaw. KCOT has been long been characterized as an odontogenic cyst, specifically an odontogenic keratocyst. In 2005, the World Health Association working group reclassified the cyst as a benign tumor for several

clinical and molecular genetic reasons (Barnes, Eveson et al. 2005). Aggressive growth patterns and high recurrence rates raised an issue regarding nomenclature as far back as the 1960s (Toller 1967). The recurrence rate in studies over the last two decades varies from 15-58%, depending on the treatment status (Myoung, Hong et al. 2001, Zhao, Wei et al. 2002, Finkelstein, Hellstein et al. 2013, Sanchez-Burgos, Gonzalez-Martin-Moro et al. 2014). Compared to KCOT, the recurrence rate of the benign but aggressive ameloblastoma is 8-38% (Reichart, Philipsen et al. 1995, Antonoglou and Sandor 2015) while that of the histologically similar orthokeratinized odontogenic cyst (OOC) is 0-6% (Li, Kitano et al. 1998, Dong, Pan et al. 2010).

Recently, molecular genetic features present in various tumors have been found in KCOTs as well. Proliferative markers proliferating cell nuclear antigen and Ki67 were expressed higher than in typical odontogenic cysts although this does not directly prove KCOT is a tumor (Li, Browne et al. 1994, Li, Browne et al. 1995, Piattelli, Fioroni et al. 1998). Tumor suppressor genes (e.g. p53, p16, MCC, FHIT and PTCH) demonstrated mutations or loss of heterozygosity in several cases, supporting the neoplastic biologic behavior of KCOT (Lombardi, Odell et al. 1995, Li, Browne et al. 1996, Agaram, Collins et al. 2004, Malcic, Jukic et al. 2008).

Among these, the PTCH gene is supposed to be important in KCOT pathogenesis. Mutation in the PTCH gene, a part of the Sonic Hedgehog (SHH) pathway, was first detected in patients with nevoid basal cell carcinoma syndrome (NBCCS), then identified in sporadic KCOTs (Lench, Telford et al. 1997, Barreto, Gomez et al. 2000, Gu, Zhao et al. 2006). Aberrant activation of the SHH pathway in both sporadic and NBCCS related KCOT has been suggested by studies of SHH-related gene mutation as well as of immunoprotein expression profiles (Ohki, Kumamoto et al. 2004, Vered, Peleg et al. 2009). Compared with PTCH gene mutation in NBCCS, sporadic KCOT has a lower mutation frequency, different distribution pattern of mutation domains, and may need a more complicated 'two hit' somatic mutation (Levanat, Gorlin et al. 1996, Vered, Peleg et al. 2009, Mendes, Carvalho et al. 2010, Guo, Zhang et al. 2013). Therefore, other gene mutations in sporadic KCOT have drawn attention despite the clear role of the PTCH gene and SHH pathway in NBCCS.

BRAF mutation, specifically the BRAF V600E mutation, has been identified in odontogenic tumors such as ameloblastoma and ameloblastic-like lesions (Brown, Rolland et al. 2014, Kurppa, Caton et al. 2014, Sweeney, McClary et al. 2014, Brunner, Bihl et al. 2015). It was originally identified in malignant tumors such as malignant melanoma, colorectal cancer and ovarian cancers (Davies, Bignell et al.

2002). Later, it was recognized in benign melanocytic nevus and suspected as a driver mutation beginning with its benign status and continuing throughout malignant alterations (Pollock, Harper et al. 2003, Yazdi, Palmedo et al. 2003, Shain, Yeh et al. 2015).

The purpose of this study was to validate BRAF V600E mutations in KCOT using formalin fixed paraffin embedded (FFPE) tissue samples and to identify a molecular pathogenetic target for non-invasive therapeutics.

II. Material and methods

1. Samples

FFPE tissue samples from 39 cases of KCOT (38 patients), 7 cases of OOC, 4 cases of ameloblastoma, and 2 cases of pyogenic granuloma were obtained from the Department of Oral Pathology at Yonsei University. Ameloblastoma samples were included to re-confirm previous reports and pyogenic granuloma samples consisting of nonspecific inflammatory granulation tissue were selected as a negative control for gene sequencing. If there were multiple block sections in a single lesion, the preferred conditions were excised, non-decalcified, non-inflamed, higher tumor fraction sections over incised, decalcified, inflamed and lower tumor fraction. All tissue samples, sectioned and stained with hematoxylin-eosin (H-E), were reviewed and diagnosed by oral pathologists E. S. C. and J. I. Y. Clinical information such as age, sex, location, recurrence status, multiple lesions status, and clinical features suggestive of NBCCS were recorded retrospectively.

2. Tumor fraction analysis and microdissection

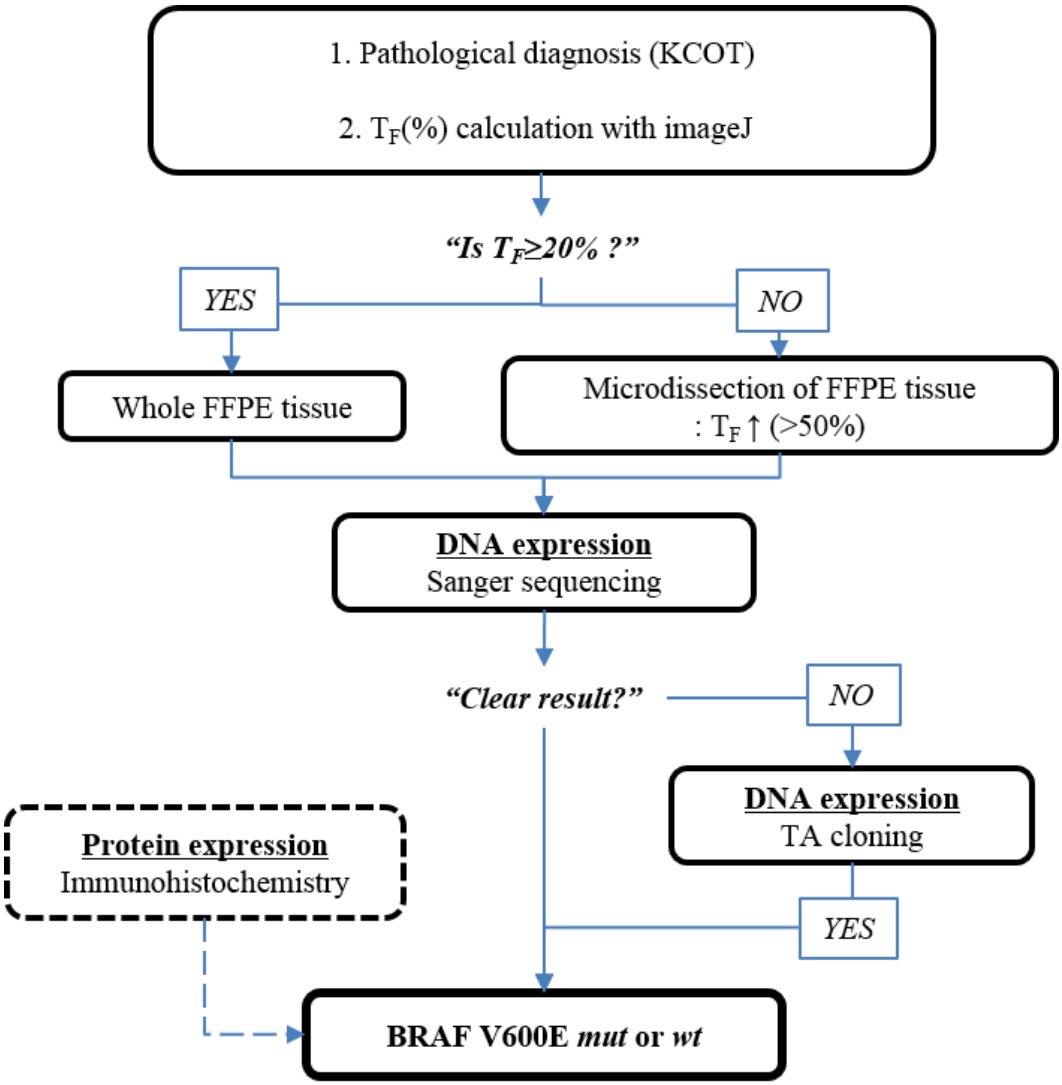
Tumor fraction was calculated in all the samples by H-E slide examination and ImageJ (1.50i, National Institutes of Health, USA) (Figure 1A). Samples with tumor

fractions less than 20% were manually microdissected until the tumor fraction exceeded 50% (Figure 1B). Microdissection of the tumor was done on a wet glass slide of sectioned unstained paraffin block with a thickness of 10㎛. Areas of heavily inflamed tissue were avoided during microdissection.

3. Genomic DNA isolation and PCR

Genomic DNA was isolated from FFPE sections (thickness 5~10㎛) by QIAamp DNA FFPE Tissue Kit (QIAGEN, cat. No. 56404) following the manufacturer's instruction manual. Paraffin was removed with xylene and the remaining tissue was bound with 100% ethanol. The contents were lysed with proteinase using lysis buffer at 56℃, 1 hour and 90℃, over 1 hour on heat block. DNA precipitation and quantification (50ng/㎕) was done. PCR using ACCUPOWER Pfu premix (50㎕ reaction, Bioneer) was done for 35 cycles (94℃ for 30 s, 58℃ for 30 s and 72℃ for 60 s). The primer sequence for BRAF (600) was forward 5'-TGCTTGCTCTGATAGGAAAATG-3' and reverse 5'-CCACAAAATGGATCCAGACA-3' as previously reported (Sweeney, McClary et al. 2014). The PCR products were verified through gel electrophoresis (1.5% agrose gel, expected product size: 173bp) and purified.

A



FFPE: Formalin fixed paraffin embedded
T_F : Tumor (cyst epithelium) fraction (%)
mut: mutant *wt*: wild type

B

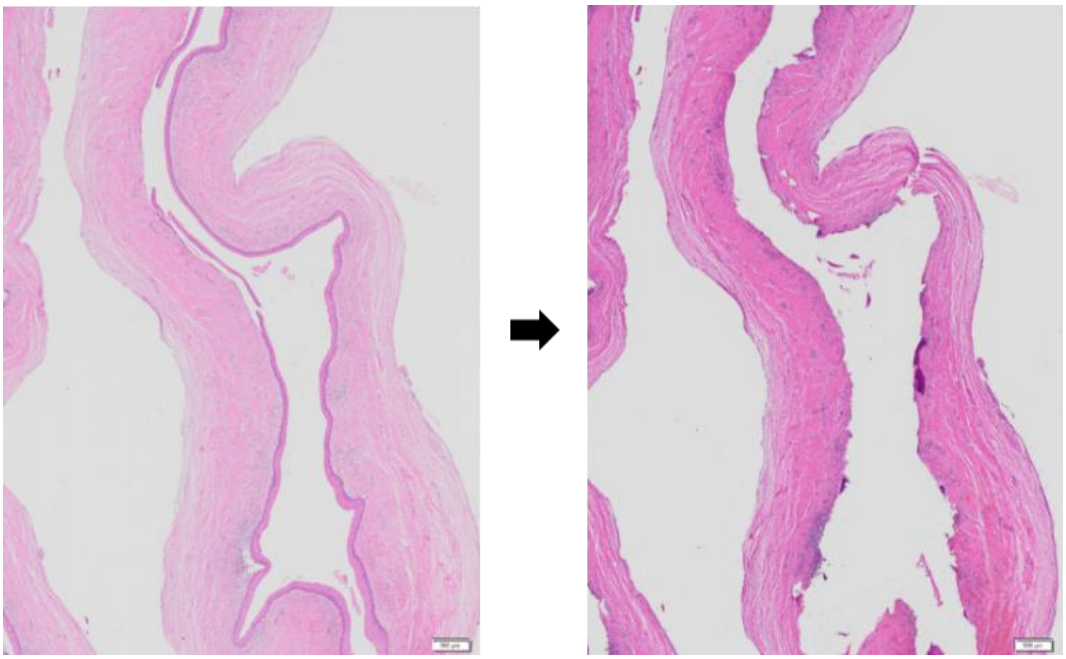


Figure 1. Identification process of BRAF V600E mutation in keratocystic odontogenic tumor (KCOT)

A. Gene sequencing with or without microdissection, rather than immunohistochemistry, is the gold standard to identify BRAF V600E mutation in KCOT.

B. Microdissection of tumor epithelium in KCOT (hematoxylin-eosin, x10, scale bar = 500 ㎛).

4. Sanger sequencing

Sanger sequencing (Macrogen) was done and review of the sequence was performed by manual examination. The sample was defined as a mutant if there was a definite sequencing peak of BRAF c.1799 T>A. Samples with indefinite sequencing peaks of BRAF c.1799 T>A mutation were clarified by taq-PCR TA cloning (T vector, Real Biotech Corp.) and blue white screening.

5. Immunohistochemistry

Immunohistochemistry was performed on 3㎛ sectioned FFPE sections with BRAF V600E mutation specific antibody at ASAN medical center (Ventana, clone VE1, 12㎍/ml and Ventana BenchMark XT immunostainer) and at Yonsei University, Department of Oral Pathology (NewEast biosciences, 1:100 and manual staining). A BRAF V600E mutated human colorectal cancer specimen was used as positive control during automated staining.

6. Statistics

Chi-square test and Fisher's exact test were performed on the association of mutation and clinical information. Recurrence-free survival was determined by the

aplan-Meier method using log-rank test. Analysis was done with SPSS statistics 21.0 (SPSS Inc, Chicago, IL, USA). The level of statistical significance was set at $p < 0.05$.

III. Results

1. Frequent BRAF V600E mutation in KCOT by gene sequencing

We collected 39 cases of KCOT (38 patients), 7 cases of OOC, 4 cases of ameloblastoma, and 2 cases of pyogenic granuloma. 2 samples of recurrent multiple KCOT were collected from the same patient and only the primary lesion was included in statistical analysis.

BRAF V600E mutations were identified in the KCOT, OOC and ameloblastoma samples (Figure 2 and 3). Mutation ratio was 63.2% in KCOT, 28.6% in OOC (Table 1), 50% in ameloblastoma and 0% in pyogenic granuloma. The 2 recurrent multiple KCOT lesion samples from the same patient were both BRAF wild type.

2. Analysis of BRAF V600E mutation status and clinical characteristics association

There was no statistically significant difference between KCOT mutation status and clinical information such as age, sex, location, recurrence and multiple lesions (Table 2). In the KCOT samples, average patient age at sample collection was 30 years old, and the male to female ratio about 3:2. 58% of the samples were from the mandible, while the rest were from the maxilla. 50% were from the right side and

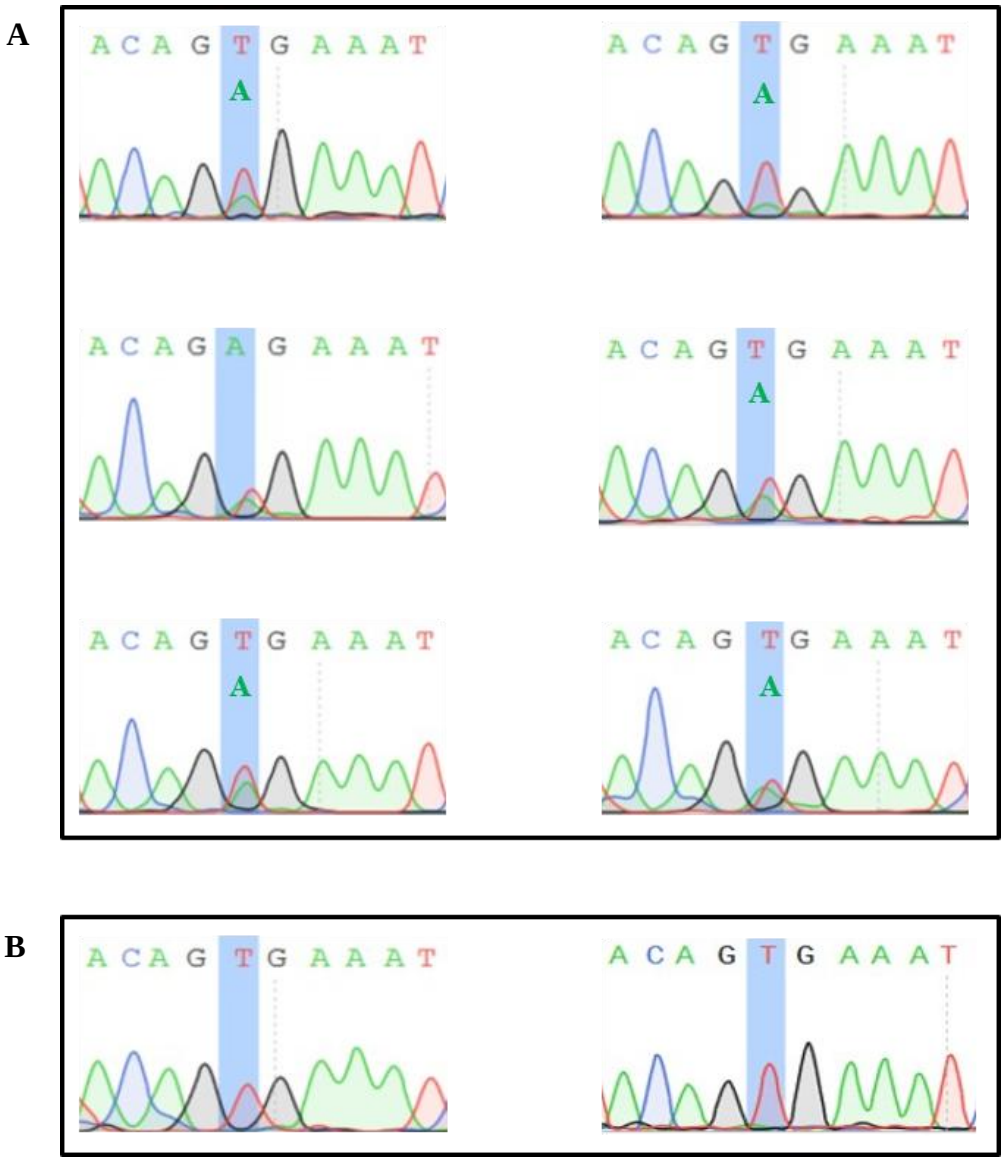


Figure 2. Frequent BRAF V600E mutation (63.2%) was identified in keratocystic odontogenic tumor (KCOT) by sanger sequencing.

A. V600E mutant KCOT samples with T>A at nucleotide 1799.

B. Wild type KCOT samples with a single T peak at nucleotide 1799.

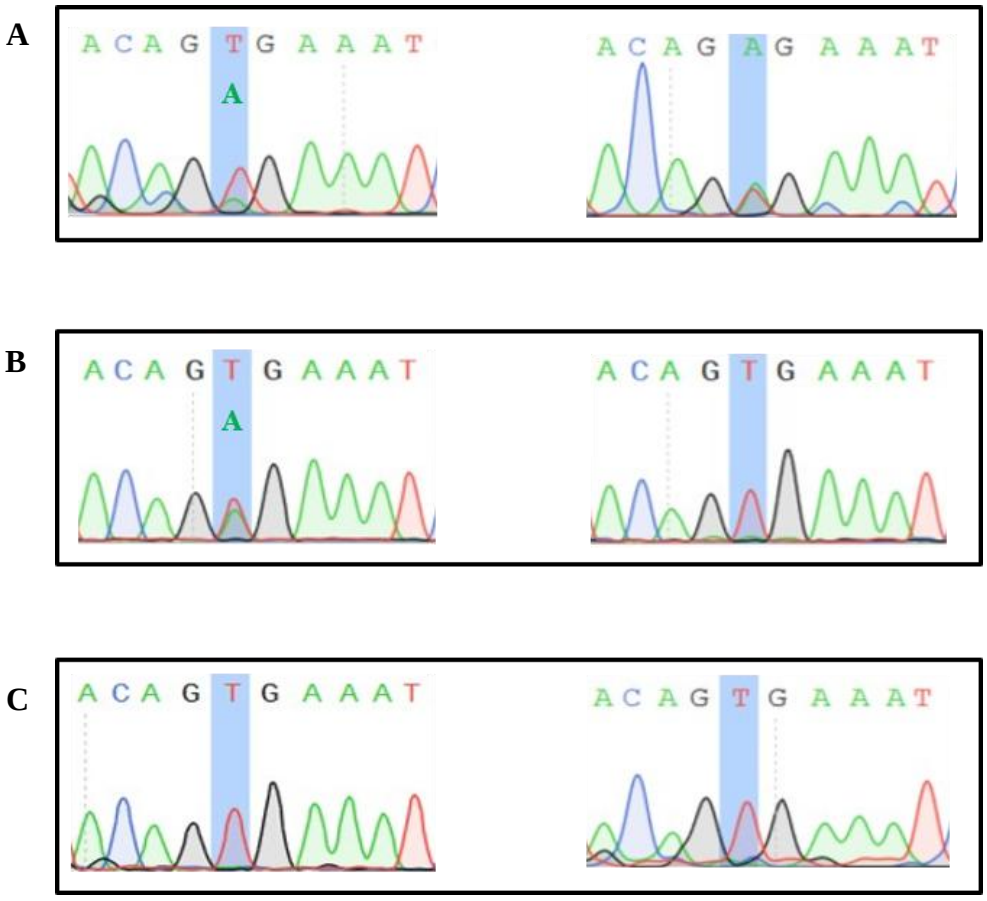


Figure 3. Gene sequencing results of orthokeratinized odontogenic cyst (OOC), ameloblastoma, and pyogenic granuloma.

A. 2 of the 7 OOC samples (28.6%) were BRAF V600E mutants.

B. 50% of the ameloblastoma samples were BRAF V600E mutants which confirmed the previous reports (Left: mutant, Right: wild type).

C. Pyogenic granuloma samples were selected as negative control and were all BRAF wild types.

Pathologic diagnosis	Patients (n)	V600E mut (n)	BRAF wt (n)	Mutation (%)
KCOT	38	24	14	63.2
OOC	7	2	2	28.6

Table 1. BRAF V600E mutation frequency in keratocystic odontogenic tumor (KCOT) and orthokeratinized odontogenic cyst (OOC). (mut: mutant, wt: wild type)

Mutation status / Clinical characteristics	Total (n=38)	V600E mut (n=24)	BRAF wt (n=14)	p value
Age at sample collection (years)	30±14	32±14	27±13	0.242
Age at initial diagnosis (years)	28±15	31±15	24±13	0.157
Sex; male	63% (24)	67% (16)	57% (8)	0.403
Sample location; maxilla	42% (16)	33% (8)	57% (8)	0.137
Sample location; mandible	58% (22)	67% (16)	43% (6)	0.137
Pt with recurrence experience	63% (24)	58% (14)	71% (10)	0.326
Pt with multiple lesions	34% (13)	25% (6)	50% (7)	0.113

Pt : patient(s)

Table 2. Clinical characteristics of BRAF V600E mutant (mut) and wild type (wt) keratocystic odontogenic tumor.

79% were from the posterior side.

63% of the total KCOT patients had experienced recurrent lesions during the follow up period, while all the OOC patients were free of recurrence. Recurrence free survival showed no statistically significant difference (p value = 0.479) between mutants and wild types (Figure 4).

34% of the patients had multiple lesions. Although most of the patients had definitive sporadic lesions, 2 of the multiple KCOT patients fit two major criteria of NBCCS such as basal cell carcinoma under 30 years and multiple palmar pitting. A patient with a single KCOT lesion occurred with one minor criterion (medulloblastoma) and was suspicious of NBCCS. Within the 3 patients who showed NBCCS-related characteristics, 2 samples with multiple KCOTs matching major criteria were mutants and a sample with single KCOT matching minor criteria was wild type (Neville, Allen et al. 2016).

All 3 samples with pre- or post-ameloblastoma occurrence and/or focal ameloblastic change were mutants and exhibited recurrence during the follow-up period. 2 samples with focal ameloblastic change in the KCOT had a history of primary or recurrent ameloblastoma and yet another typical sample later recurred into a KCOT with ameloblastic change during follow-up.

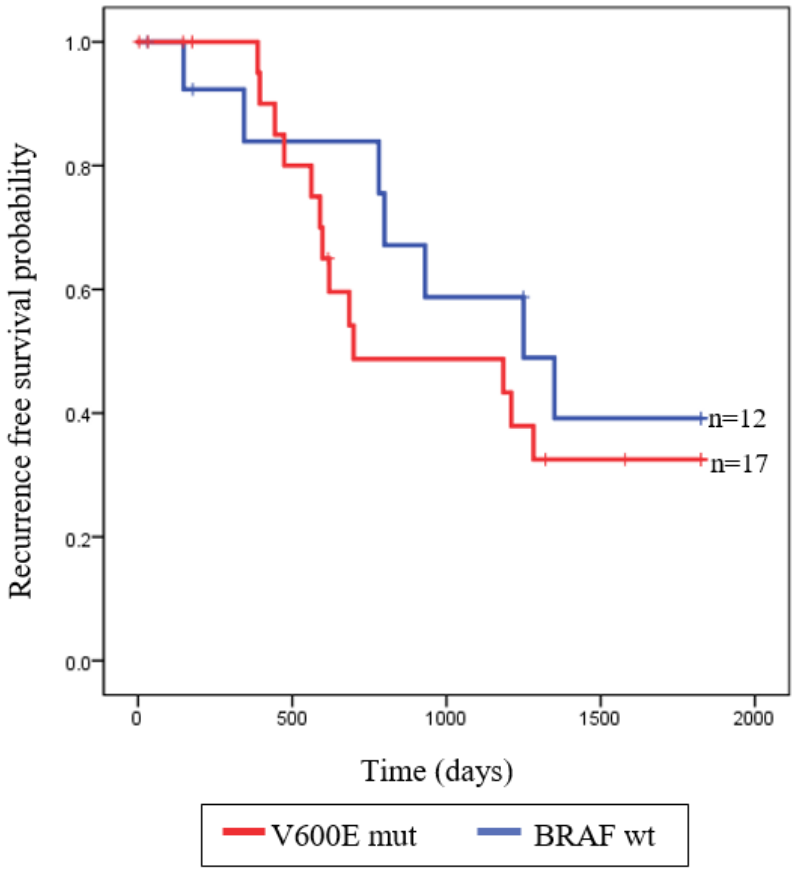


Figure 4. 5 year follow up recurrence free survival (in days) for BRAF V600E mutant (mut) and wild type (wt) keratocystic odontogenic tumor (KCOT) patients using Kaplan-Meier method.

Patients with recurrent event(s) within 5 years from the first treatment regardless of follow up period, or patients without recurrent event(s) fulfilling the 5 year follow up were included for analysis (n=29).

3. Negative BRAF immunohistochemistry results in KCOT

Immunohistochemistry results were negative in all of the KCOT samples (Figure 5). Colorectal cancer used as a positive control stained strong, diffusely staining at the tumor cell cytoplasm, verifying the staining process. Ameloblastoma showed a weak stain on the tumor cells but staining results did not fully correlate with the genetic status.

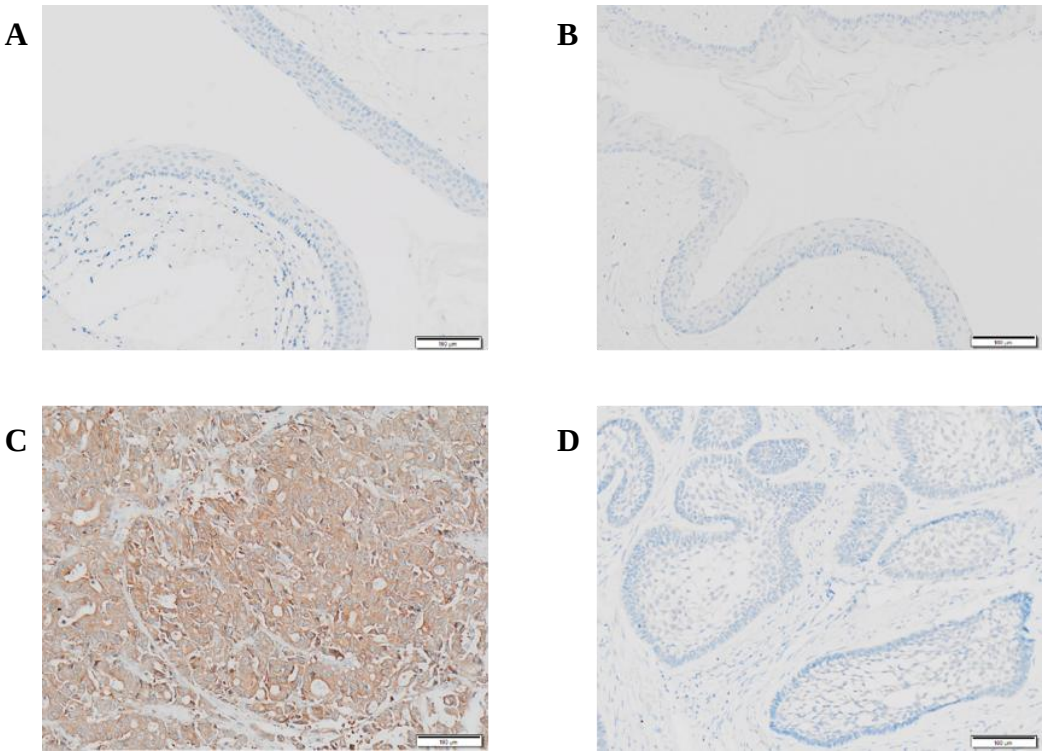


Figure 5. Negative BRAF immunohistochemistry results (VE1 clone)

Both keratocystic odontogenic tumor (KCOT) mutant sample (A, x100) and KCOT wild type sample (B, x100) did not show positive staining in the tumor cells. A colorectal cancer sample with BRAF V600E mutation (C, x100) was used as positive control. Ameloblastoma mutant sample showed a diffuse weak cytoplasmic staining in the tumor cells and negative staining results in the stromal cells (D, x100) (scale bar = 100 ㎛).

IV. Discussion

BRAF V600E mutations were identified for the first time in KCOT as well as OOC. BRAF mutations are known to activate the mitogen-activated protein kinase (MAPK) pathway, resulting in abnormal cell proliferation and survival. Among BRAF mutations, V600E is the most common type, with valine substituted for glutamic acid at codon 600 in exon 15 (Davies, Bignell et al. 2002, Hertzman Johansson and Egyhazi Brage 2014). KCOT showed a high frequency of mutants, similar or slightly higher than the mutation rate previously reported in ameloblastoma (46-63%) (Brown, Rolland et al. 2014, Kurppa, Caton et al. 2014, Sweeney, McClary et al. 2014), while OOC showed a much lower frequency of mutants. Although OOC was once treated as an orthokeratinized variant of KCOT, it has recently come to be thought of as a distinct type of odontogenic cyst while KCOT has been reclassified as a neoplasm (Li, Kitano et al. 1998, Dong, Pan et al. 2010). Interestingly, all the samples with pre- or post-ameloblastoma occurrence and/or focal ameloblastic change were mutants. This suggests that KCOT and ameloblastoma share certain parts of their genetic pathogenesis and supports KCOT as a tumor. BRAF mutation was identified in both sporadic and syndrome associated KCOTs. Samples in this study with definite diagnostic criteria of NBCCS were all mutants. Although genetic confirmation of NBCCS was not

medically concluded in the patients, BRAF mutation was suspected to play a role in syndrome-associated KCOT pathogenesis.

A previous study has failed to confirm BRAF mutation in KCOT in 1 case (Brunner, Bihl et al. 2015). Besides the insufficient case number of the study, identifying BRAF mutations in FFPE tissues of KCOT requires an attentive approach. Cystic tumors have a relatively low tumor fraction compared to solid tumors. Furthermore, cystic odontogenic lesions with a history of marsupialization or decompression accompany dense inflammatory cell infiltration and increased stroma. Dense nonspecific inflammation decreases tumor fraction and may mask samples as wild type rather than mutant. Microdissection was introduced in this study to increase tumor fraction and reveal mutants hidden behind inflammation and thick stromal tissue. This method may be useful in validating mutations in cystic tumors or inflamed lesions.

Immunohistochemistry was done multiple times, both by automatically and manually, but never revealed positive protein expression in the cyst epithelial tumor cells. Colorectal cancer, a positive control, expressed definite positive cytoplasmic staining, which indicated that the staining process was technically acceptable. As a point mutation, BRAF V600E may exhibit protein expression too low to detect with immunohistochemistry (Capper, Preusser et al. 2011); the gold

standard in validating the mutation is gene sequencing with or without microdissection (Figure 6).

Correlation between BRAF V600E mutant status and clinical information could not be proven in KCOT. Ameloblastoma studies report correlations between BRAF V600E mutant status and age, location, and recurrence-free survival, (Brown, Rolland et al. 2014, Sweeney, McClary et al. 2014, Fregnani, da Cruz Perez et al. 2016), results not found in this study of KCOT. Because BRAF wild type tumors likely bear other oncogenic mutations which may influence the mentioned factors, it is understandable that studies would yield different results with respect to these correlations. Further research is needed to validate the complete oncogenic mutation profile in KCOT.

In KCOT, therapeutics additional to surgery may be an effective alternative due to various complications in surgery and its high recurrence rate. Brannon suggested that the high recurrence rate of KCOT was due to epithelial remnants adjacent to the former lesion, incomplete removal of the friable epithelial lining, and satellite cysts (Brannon 1977). Traditional treatment options are enucleation, excision, ostectomy and en bloc resection (Sharif, Oliver et al. 2015). KCOT most frequently occurs in the second and third decade and often is multilocular (Brannon 1977, Myoung, Hong et al. 2001), so surgical treatment planning with an esthetic focus

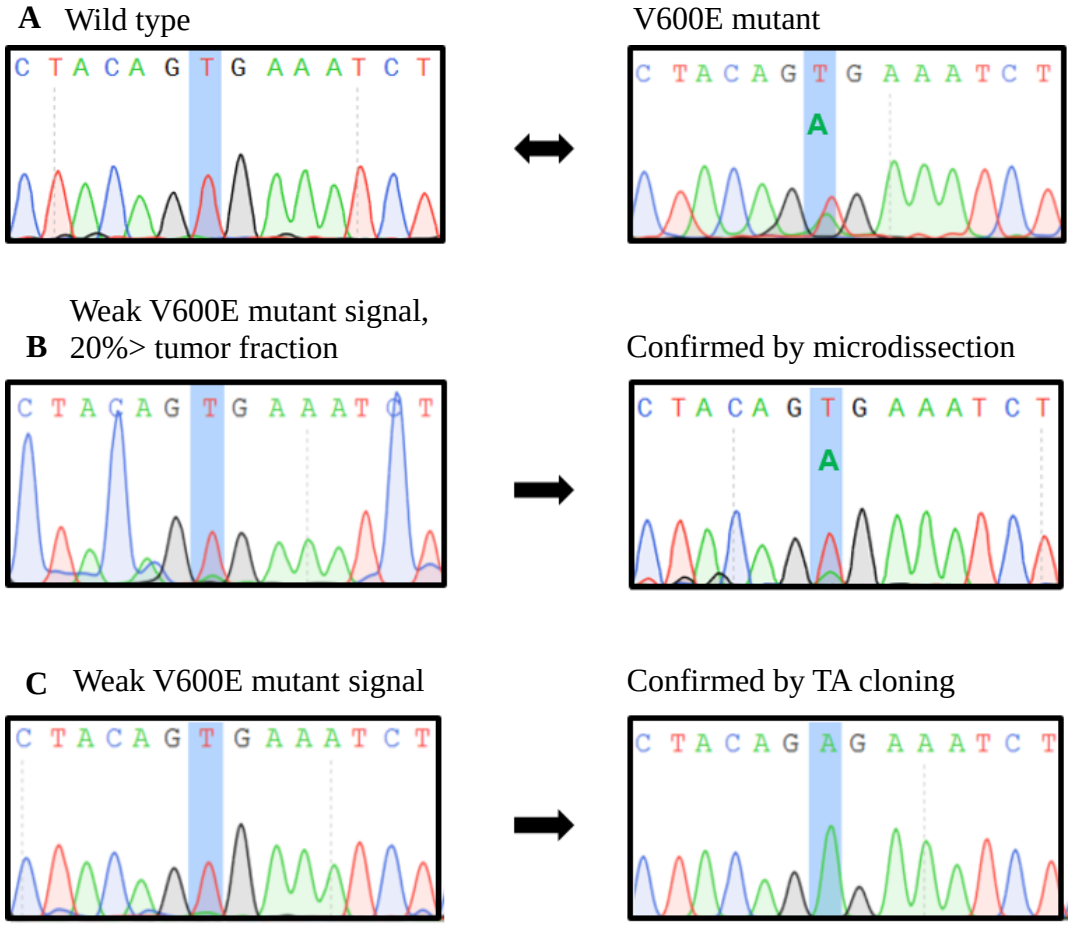


Figure 6. Methods to increase accuracy of gene sequencing results in keratocystic odontogenic tumor.

A. General PCR amplification and sanger sequencing

B. Microdissection in samples with less than 20% tumor fraction resulted in a stronger A nucleotide signal, confirming mutation.

C. Samples with weak V600E mutation signals were confirmed with TA cloning.

may encounter limitations.

Inhibitors targeting BRAF mutations and the MAPK pathway may play a potential role in KCOT with BRAF mutations. BRAF inhibitors vemurafenib and dabrafenib are United States Food and Drug Administration (FDA) approved drugs for metastatic melanoma (Chapman, Hauschild et al. 2011, Menzies and Long 2014). Drug resistance is a problem when treating malignancies with BRAF inhibitors because of constant additional genetic alterations which make full recovery difficult (Hertzman Johansson and Egyhazi Brage 2014). Effective therapeutic results are expected in benign tumors due to their simpler genetic mutation profile (Yazdi, Palmedo et al. 2003, Michaloglou, Vredeveld et al. 2008, Shain, Yeh et al. 2015). KCOT recurrence is frequent because of its satellite cysts and cyst epithelium remnant after surgery. Pre- or post-operative use of BRAF inhibitors may control the growth of the tumor remnants and potentially constrain the aggressive biological behavior, decreasing the total range of surgery and recurrence rate.

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VI. Supplementary tables

No	Age	Sex	Location	Treatment	Recurrence status	No. of lesions	BRAF status	T_F (%)	other
Keratocystic odontogenic tumor (KCOT)									
1	37	f	Right posterior mandible	enucleation	Primary without recurrence	single	V600E	30	
2	54	f	Left posterior maxilla	enucleation	Primary without recurrence	single	V600E	20	
3	33	m	Left posterior maxilla	enucleation	3rd in 3 recurrences	single	V600E	20	
4	31	m	Left posterior mandible	enucleation	Primary without recurrence	single	V600E	50	
5	32	m	Left posterior mandible	enucleation	2nd in 2 recurrences	single	V600E	50	
6	20	m	Left posterior maxilla	enucleation	Primary without recurrence	single	wt	20	
7	38	m	Right posterior maxilla	enucleation	Primary without recurrence	single	V600E	10>	
8	50	m	Right anterior mandible	enucleation	1st in 1 recurrence	single	wt	20	
9	35	m	Left posterior mandible	enucleation	Primary without recurrence	single	V600E	20	
10	22	f	Left posterior mandible	enucleation	Primary without recurrence	single	V600E	20	
11	59	m	Left posterior mandible	enucleation	Primary without recurrence	single	V600E	10	
12	34	m	Right posterior mandible	enucleation	1st in 1 recurrence	single	wt	60	
13	29	f	Left posterior mandible	enucleation	Primary without recurrence	single	wt	30	
14	52	f	Left posterior mandible	curettage	Primary in 1 recurrence	single	wt	20	
15	10	m	Right posterior maxilla	enucleation	Primary in 1 recurrence	single	wt	30	medulloblastoma
16	20	m	Left posterior mandible	enucleation	Primary in 1 recurrence	single	V600E	10	
17	36	m	Right	enucleation	Primary in 1	single	wt	30	

			posterior mandible		recurrence				
18	12	f	Right maxilla	enucleation	Primary in 2 recurrences	single	V600E	10>	
19	69	m	Right anterior mandible	enucleation	Primary in 2 recurrences	single	V600E	30	
20	31	m	Left posterior mandible	enucleation	Primary in 2 recurrences	single	V600E	10	
21	33	m	Right anterior maxilla	enucleation	Primary in 2 recurrences	single	V600E	20	
22	33	m	Left posterior mandible	enucleation	Primary in 2 recurrences	single	V600E	50	
23	49	m	Right posterior mandible	enucleation	Primary without recurrence	single	V600E	10>	
24	15	f	Right posterior maxilla	enucleation	Primary without recurrence	multiple	wt	10	
25	32	f	Left posterior mandible	enucleation	Primary without recurrence	multiple	V600E	40	palmar pitting, epidermal cyst
26	13	m	Left posterior maxilla	enucleation	Primary in 1 recurrence	multiple	V600E	30	
27	16	f	Right posterior mandible	incision	Primary without recurrence	multiple	V600E	10>	
28	23	m	Right posterior maxilla	enucleation	2nd in 2 recurrences	multiple	wt	10	
29	13	m	Left posterior maxilla	enucleation	Primary in 2 recurrences	multiple	wt	10	
30	15	m	Right anterior maxilla	enucleation	2nd in 2 recurrences	multiple	V600E	10	palm basal cell carcinoma
31	20	f	Right posterior maxilla	enucleation	Primary*	multiple	V600E	40	
32	28	f	Left maxilla	enucleation	Primary in 2 recurrences	multiple	wt	30	Same person with no.33
33	33	f	Right posterior mandible	enucleation	2nd in 2 recurrences	multiple	wt	20	Same person with no.32
34	14	f	Right anterior maxilla	enucleation	1st in 1 recurrence	multiple	wt	30	
35	34	f	Left posterior maxilla	enucleation	Primary*	multiple	V600E	30	
36	15	m	Left posterior mandible	enucleation	Primary without recurrence	multiple	wt	10	
37	32	f	Right	enucleation	Primary*	multiple	wt	10	

			posterior maxilla						
38	33	m	Right posterior mandible	enucleation	2nd in 2 recurrences	single	V600E	20	Uncystic ameloblastoma arised before KCOT
39	18	m	Right anterior mandible	enucleation	1st in 1 recurrence	single	V600E	60	Recurred as ameloblastoma
Orthokeratinized odontogenic cyst (OOC)									
40	32	m	Right posterior mandible	enucleation	Primary	single	V600E	20	
41	17	f	Right posterior mandible	enucleation	Primary	single	wt	50	
42	56	f	Right posterior mandible	enucleation	Primary	single	wt	20	
43	16	f	Left posterior maxilla	enucleation	Primary	single	wt	60	
44	27	f	Right posterior mandible	enucleation	Primary	single	wt	20	
45	34	m	Left posterior mandible	enucleation	Primary	single	wt	40	
46	32	m	Left posterior mandible	enucleation	Primary	single	V600E	50	

Primary*: Primary lesion in multiple KCOT; Current location is not associated with recurrence but other locations may have had recurrent events.

T_F: Tumor (cystic epithelium) fraction, V600E: BRAF V600E mutant, wt: wild type

Supplementary table 1. Clinical information and BRAF mutation status of keratocystic odontogenic tumor (KCOT) and orthokeratinized odontogenic cyst (OOC) patients

No	Diagnosis	Age	Sex	Location	Treatment	BRAF status
47	**Ameloblastoma**	40	m	Left posterior mandible	excision	wt
48		58	m	Left anterior mandible	excision	wt
49		26	f	Right posterior mandible	excision	V600E
50		22	m	Right mandible	biopsy	V600E
51	**Pyogenic granuloma**	51	f	Buccal mucosa	excision	wt
52		37	f	Left mandible	excision	wt

V600E: BRAF V600E mutant, wt: wild type

Supplementary table 2. Clinical information and BRAF mutation status of ameloblastoma and pyogenic granuloma patients

ABSTRACT (IN KOREAN)

각화낭성치성종양에서

높은 빈도로 발생하는 BRAF V600E 유전자 변이

<지도교수 최종훈>

연세대학교 대학원 치의학과

조은애산드라

각화낭성치성종양은 악골의 양성 골내 치성 상피 종양으로 과거 치성 각화낭이라는 명칭의 치성낭으로 분류되었다. 세계보건기구는 2005년에 각화낭성치성종양을 낭에서 종양으로 재정의를 하였다.

임상적으로 각화낭성치성종양은 공격적인 성장 양상과 상대적으로 높은 재발율을 보이는데, 이는 치성낭보다 치성종양의 특성에 가깝다. 분

자유전학적인 관점에서도 종양의 특성을 보이는데, 증식 관련 표지자와 종양억제유전자변이/이형접합성상실 등이 그 예이다. 이중 PTCH 유전자 변이가 가장 중요하게 여겨졌으나 기저세포모반증후군과 관련된 병소와 달리 산발적으로 발생하는 병소에서는 낮은 변이 비율 등 근거가 상대적으로 뚜렷하지 않아 다른 종양성 유전자 변이를 발견할 필요성이 있다.

최근에 BRAF V600E 유전자 변이는 법랑모세포종을 비롯한 치성종양에서 발견이 되었다. 이는 종양 유발 유전자변이로 mitogen-activated protein kinase (MAPK) 경로를 활성화하며 베무라페닙과 다브라페닙과 같은 상용화된 억제제 약물이 있다.

이 연구에서는 각화낭성치성종양에서 높은 빈도로 BRAF V600E 유전자 변이가 발생한다는 것을 유전자 시퀀싱을 통해 관찰하였다. 연구결과는 다음과 같다.

1. 포르말린 고정 조직에 유전자 시퀀싱을 하여 최초로 63.2%의 각화낭성치성종양과 28.6%의 진성각화치성낭에서 BRAF V600E 유전자 변이를 관찰되었다.

2. 각화낭성치성종양의 BRAF 유전자 변이 여부와 임상적 양상 (연령, 성별, 발병 위치, 재발 여부 및 다발성 병소 여부 등)은 통계학적으로 유의한 연관성을 보이지 않았다.

3. 각화낭성치성종양에서 BRAF V600E를 확인하기에 가장 적합한 방법은 면역조직화학염색보다 유전자 시퀀싱이다.

4. 낭성 종양 또는 염증이 심한 조직에서는 현미해부를 통해 유전자 변이 여부를 더 정확하게 확인 할 수 있다.

이러한 연구 결과들은 각화낭성치성종양이 종양임을 다시 한번 확인 시켜주며 BRAF V600E 유전자 변이 및 MAPK 경로와 연관된 병인을 가지고 있음을 보여준다. 이는 각화낭성치성종양의 치료로 표적 억제제를 이용한 비침습적 치료의 가능성을 제시하여 준다.

핵심어: 각화낭성치성종양, BRAF V600E, mitogen-activated protein kinase pathway, 베무라페닙, 다브라페닙